

Bugs in Bugs: The Microbial Diversity of the Termite Gut

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The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is an invasive pest species that causes billions of dollars worldwide in economic damage, especially in the southeastern United States and the Pacific Rim. The survival of this termite is dependent on its microbial gut community (Eutick et al. 1978). The gut flora consists of three species of protists and an unknown number of bacteria species, which play a key role in physiology, nutrition and gut ecology of the termite host (Lai et al. 1983, Bignell 2000). Because *C. formosanus* depends on its gut flora for survival, alternative termite control strategies could be devised using the gut flora of termites as tools and targets for termite control. However, before such strategies can be developed, detailed knowledge about the microbial diversity in the termite gut is sorely needed.

In this study, we used culture-independent 16S rRNA gene sequencing in conjunction with classical culture methods to describe the microbial diversity in the gut of *C. formosanus*, i.e., number and proportion of bacteria taxa, and the dominant bacteria groups. The 16S rRNA gene sequences of the total bacteria community were obtained through extraction of the bacterial communities' total DNA, PCR amplification of 16S rRNA genes using bacteria specific primers (e.g., Liu et al. 1997), making a clone library, sequencing a subset of clones and comparing the results to public databases such as GenBank. To date, 96 clones from two pools of 100 workers from two termite colonies (New Orleans, La.) were sequenced. Clones could be assigned to 12 different bacteria strains from four different groups (*Bacteroides*, *Treponema*, *Spirochaeta*, Clostridiaceae) based on the closest match in GenBank and sequence similarity to each other. *Bacteroides* was the dominant group, comprising over 75% of the gut flora in both colonies, followed by spirochetes (*Treponema* spp. and *Spirochaeta* spp.). The bacteria taxa identified in the gut of *C. formosanus* using culture-independent 16S rRNA gene sequencing were different from the bacteria we were able to culture from the gut of the same species. To date, over 20 strains of bacteria have been cultured, including species belonging to the Enterobacteriaceae, Bacteroidales and Lactobacillales. The lactic acid metabolites of the latter group are considered especially important for the ecological balance in the termite gut (Bauer et al. 2000). Potrikus and Breznak (1981) have shown that lactic acid bacteria recycle carbon and nitrogen via metabolizing uric acid. Also, several species of Enterobacteriaceae, including *Enterobacter cloacae*, *Citrobacter amalonaticus*, *Citrobacter* sp., *Klebsiella pneumoniae*, *Kluyvera* sp., and several unknown Enterobacteriaceae genera were isolated from termite guts. Satellite bacteria were frequently found growing around Enterobacteriaceae colonies. These were identified as *Dysgonomonas* spp. (Bacteroidales). Other strains were found among the minor isolates, including *Aeromonas* sp. (Aeromonadaceae), *Acinetobacter* sp. (Moraxellaceae) and unknown lactic acid bacteria. Several strains had the capability to grow on minimal media (without a nitrogen source) and thus were likely nitrogen fixers. The functional group of nitrogen fixing bacteria is necessary to supplement the nitrogen-poor diet of the termites. We are in the process of describing new bacteria species and genera (Higashiguchi et al. in press) and 16S rRNA gene sequences have been submitted to GenBank. All of the species identified by their 16S rRNA gene sequences and most of the cultured strains were novel

species found exclusively in the termite gut.

The knowledge gained from this study is expected to spark new ideas and products for termite management employing the termites' gut flora in the future. A current project uses bacteria isolated from the termite gut as "Trojan Horses" to deliver and spread foreign genes through termite colonies (Husseneder and Grace in press).

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